TITLE: MOLECULAR REGULATORS OF HAB GROWTH AND TOXICITY

MILESTONE SHC 2.5.2: Transfer technology, tools, and knowledge related to harmful algal bloom events to assist communities, resource managers, and health officials.
NCCOS ID: NCCOS13
CENTER ID: CCEHBR30

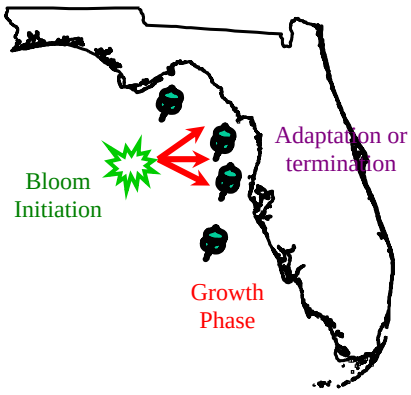
CCEHBR SCIENTISTS/INVESTIGATORS: Frances Van Dolah (PI), Michele Barbier, Bennie Haynes, Tod Leighfield, Jeanine Miller, Jeral Tyler

EXTERNAL COLLABORATORS: Florida Marine Research Inst., North Carolina State Univ., Mote Marine Laboratory,

OBJECTIVES OF RESEARCH ACTIVITIES: Identify molecular regulators of harmful algal growth and cell division, toxigenicity, stress responses and cell death, and evaluate their potential to be exploited as a means to terminate bloom events and reduce risks to coastal resources and communities.

DESCRIPTION OF RESEARCH ACTIVITIES: Algal growth regulation studies currently focus on *Karenia brevis*, the Florida red tide dinoflagellate. Biochemical pathways critical to two phases of bloom formation are being addressed in two projects:

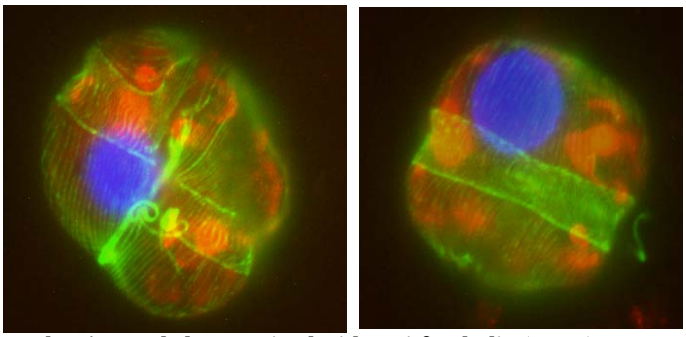
1. The cell cycle of many dinoflagellates is controlled by a circadian rhythm entrained to the diel cycle, which serves as a rate-limiting regulator of the growth of harmful algal blooms. This project thus focuses on molecular mechanisms that control the dinoflagellate cell cycle and the signaling pathways that link the cell cycle to the diel cycle. As part of this project, large scale screening of a cDNA library has been undertaken to provide tools to investigate gene expression.
2. Blooms of *K. brevis* become a threat to coastal ecosystems and humans only when carried inshore by prevailing winds and currents. There they may persist for many months within coastal embayments, or they may terminate rapidly. The processes that result alternatively in cellular adaptation and bloom persistence, or cell death and bloom termination, may be suitable targets for developing predictive indicators or control strategies. Therefore, in this project characterizes stress proteins in *K. brevis* involved in mediating cellular adaptation to coastal waters and investigates the involvement of programmed cell death in bloom termination.



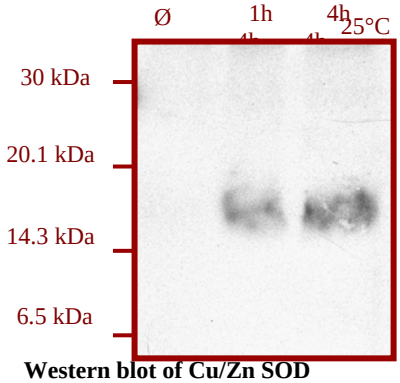
Selected Highlights from FY02

Several important achievements were completed in FY02:

- Large scale sequencing of a *K. brevis* cDNA library initiated in FY02 has yielded approximately 1150 sequences for expressed genes (ESTs) that have yielded tools for investigating gene expression.
- Identification of several cell cycle genes involved in S-phase of *K. brevis* and initial characterization of their expression, including PCNA, ribonucleotide reductase, single strand DNA binding protein, DNA ligase.
- Characterization of the tubulin cytoskeleton of *K. brevis* and its behavior throughout the cell cycle.
- Identification and characterization of the induction of heat shock proteins (Hsp60, chloroplasts sHSP, mitochondrial sHSP) and oxidative stress proteins (Mn SOD, Cu/Zn SOD) in *K. brevis* in response to heat and oxidative stress.



***K. brevis* cytoskeleton stained with anti-β-tubulin (green), nucleus stained with DAPI (blue), and chloroplasts (red).**



Western blot of Cu/Zn SOD induction after heat stress at 30°C.

Publications/Reports:

Barbier, M., Miller, J., Leighfield, T., Van Dolah, F. (accepted) Expression of α-,β-,and γ–tubulin, the minimal set of tubulins required to define microtubule function in eukaryotic cells, in the unicellular dinolfagellate, *Karenia brevis.* Phycologia.

Barbier, M, Leighfield, TA, Soyer-Gobillard, M-O., and Van Dolah, F.M. (accepted) Expression of a cyclin B homologue in the cell cycle of a primitive dinoflagellate, Karenia brevis. J. Euk. Microbiol.

Leighfield, TA, Barbier, M, and Van Dolah, FM (2002) Evidence for cyclic AMP dependent protein kinase in the dinoflagellate, *Amphidinium operculatum.* Comp. Biohem. Physiol. *B. (in press)*

Presentations:

Miller, J. and Van Dolah, F.M. Characterization of stress proteins in the Florida red tide, *Karenia brevis*. Southeastern Phycological Colloquium, Wilmington, NC, Oct 12-13, 2001.

Barbier, M. and Van Dolah, F.M. Expression of cyclin in the dinoflagellate, *Karenia brevis*. Southeastern Phycological Colloquium, Wilmington, NC, Oct 12-13, 2001.

Van Dolah, F.M., Barbier, M. Leighfield, T., Tyler, G., Haynes, B. Control of cell division in the Florida red tide, *Karenia brevis*: a genomic approach. SC Research Conference, Charleston, SC, Jan. 4, 2002.

Van Dolah, F.M., Barbier, M. Leighfield, T., Tyler, G., Haynes, B. Growth regulation in the Florida red tide, *Karenia brevis*: a genomic approach. ASLO, Victoria, B.C. June 10-14, 2002